SeMNPV reactivation through stress factors in covertly infected *Spodoptera exigua*

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Abstract: The aim of this study is to evaluate the effect of different stress factors on covertly infected *Spodptera exigua* larvae in terms of virus reactivation. For this, adult survivors that had ingested occlusion bodies of *S. exigua* multiple nucleopolyhedrovirus (SeMNPV) were mated and the subsequent generation (F_1) tested for virus reactivation in the second instar both in laboratory and field conditions. In the laboratory a number of treatments were tested including chemical stressors, inoculation with permissive and non permissive NPV species and *Bacillus thuringiensis* spores and crystals. Both, parental and F_1 adults were confirmed to harbor the infection by qPCR. Reactivation was observed in 0.1% copper sulfate, 1% iron sulfate, and 1 ppm sodium selenate treatment that resulted in 12, 15, and 41% mortality due to SeMNPV, while no larvae with symptoms of viral infection were registered in virus-free controls. No effect on NPV-induced mortality was detected after inoculation with heterologous virus. Lately field trials were carried out by artificial infestation of pepper crops in experimental greenhouses using sublethally infected *S. exigua* larvae to evaluate copper sulfate and sodium selenate as activation factors. Hardly any NPV-induced mortality was observed in those larvae treated in field conditions.

Key words: NPVs reactivation, stress factors, *Spodoptera exigua* multiple nucleopolyhedrovirus

Introduction

Recently studies on baculovirus transmission reported a high prevalence of sublethal or covert infections in lepidopteran populations such as *Spodoptera exigua* (Cabodevilla *et al*., 2011a). Spontaneous nucleopolyhedrovirus (NPV) outbreaks might be the underlying phenomena explaining natural epizooties in host population. However very little is known about the mechanisms thereby covert infections became into patent fatal infections. Virus reactivation has been related to stress conditions for larvae submitted to high densities of rearing (Fuxa *et al*., 1999), extreme temperatures or certain chemicals treatments (Ilyinykh *et al*., 2004). Investigating on factors involved in virus reactivation may contribute to the development of new strategies of biological control using NPV-based biopesticides. The aim of this study is to evaluate the effect of different groups of treatments as activation factors on covertly infected *S. exigua* larvae both in laboratory and field conditions.

Material and Methods

Insect and virus

A virus-free colony of *S. exigua* maintained in the insectary of Universidad Publica de Navarra was used for this experiment. The VT-SeAl1 strain of SeMNPV was used to inoculate *S. exigua* larvae. This strain was characterized previously by Cabodevilla et al., 2011a.

Covert infection induction and qPCR virus detection

Covert infections were established in *S. exigua* virus-free cultures according to the methods describe by Cabodevilla *et al*., (2011b). Briefly, L_4 virus-free larvae were sublethally infected with the vertically transmitted isolate VT-SeAl1. A group of larvae were dosed similarly except that the inoculum did no contain the virus, this lineage was used as control. Adult survivors to the virus challenge were mated and the subsequent generation (F_1) tested for virus reactivation on second instars by exposition of the covertly infected larvae to both chemicals and entomopathogens. Groups of 24 larvae were dosed by droplet-feeding with one of the following groups of treatment: i) chemical stressors 1%-0.1% copper sulfate, 1% iron sulfate, 1-0.1% hydroxylamine, 2% Tinopal, 1ppm sodium selenate, 1 ppm paraquat dichloride; ii) inoculation with: *Chryxodeisis chalcites* NPV (non-permissive), *Mamestra brassicae* NPV (permissive), SeMNPV-US2, *Bacillus thuringiensis* spores, *Bt* crystal, mixed *Bt* spores & crystals (1:1); and iii) rearing temperatures of 18 ºC and 28ºC NPV mortality was registered by checking cadavers for OB presence under phase-constrast microscope. To confirm the virus transgenerational transmission a group of F_1 larvae were reared to adults and tested for amplification of the viral specific gene *DNA polymerase* by qPCR using a SYBR based method as described by Cabodevilla et al (2011b).

Field trials

To determine the capacity of reactivation of those chemicals that had been proved as reactivation factors in the laboratory experiments we tested them in field conditions. Three 100 m^2 independent experimental greenhouses of IFAPA (Instituto de Investigación y Formación Agraria y Pesquera, Almeria, Spain) were set up with pepper crops using a plantation frame of 0.5 × 1 m. Each greenhouse was split into four plots in which one of the four treatments was randomly disposed: i) 0.1% copper sulfate, ii) 1 ppm sodium selenate, iii) Bt-based insecticide (FlorBac, BAYER), and iv) water control. The offspring from sublethal infected adults obtained as described above (100% positive for qPCR) were used for artificial infestations. Egg masses were displayed in the three central plants of each plot to the equivalent of 200 eggs per plants. Once most of the larvae reached second instars, aqueous dilutions of treatments were applied using a handle sprayer on the pepper plants previously infested. After 48 h post-treatment a total of 30 larvae per plot were collected from the three central plants and confined individually in 25 ml plastic cups provided with diet and reared in standard conditions until death or pupation.

Results and Discussion

Reactivation of SeMNPV by stressor factors in laboratory conditions

A hundred percent of F_1 tested adults (n=27) were confirmed to harbor the virus by qPCR, suggesting a high prevalence of the persistent infection in larvae subjected to activators treatment. NPV mortality was observed in 0.1% copper sulfate, 1% iron sulfate, and 1 ppm sodium selenate treatments that resulted in 12, 15, and 41%, while no larvae with symptoms of viral infection were registered in virus-free controls (Table 1). Ilyinkykh *et al*., (2004) reported similar results on activation of occult virus by feeding *Lymantria dispar* larvae on

diet with 0.6% of copper sulfate. Copper iron and selenium are essential microelements required for body functions and the immune system well being (Chaturvedi *et al*., 2006). They have been described as involved in inmunomodulation that influence the course of the outcome of a variety of viral infections. Therefore the chemicals used here that result in activation of the virus seems to be acting as physiological stressor.

Table 1: NPV induced mortality and mortality due to other causes in virus-free and covertly infected L_2 *S. exigua* larvae after treatment with stressor.

	Virus -Free		Covertly- infected	
Treatment	**Other causes (n)**	**NPV Mortality (n)**	**Other cuases (n)**	**NPV Mortality (n)**
Chemicals				
$CuSO_4$ (1%)	35 (37)	0 (37)	6 (37)	0 (37)
$CuSO_4$ (0.1%)	0 (23)	0 (23)	0 (58)	12 (58)
$FeSO_4$ (1%)	0 (24)	0 (24)	0 (71)	15 (71)
NH_2OH (1%)	25 (4)	0 (4)	100 (4)	0 (4)
NH_2OH (0.1%)	0 (24)	0 (24)	0 (63)	0 (63)
Tinopal (2%)	0 (19)	0 (19)	0 (72)	0 (72)
Na_2SeO_4 (1ppm)	2 (24)	0 (24)	2 (61)	41 (61)
Paraquat (1 ppm)	0 (20)	0 (20)	0 (70)	0 (70)
Entomopathogenes				
ChchNPV (1×10^8 OBs/ml)	0 (23)	0 (23)	0 (67)	0 (67)
MbNPV (1.3×10^4 OBs/ml)	0 (22)	50 (22)	0 (64)	64 (64)
SeMNPV (4.9×10^4 OBs/ml)	0 (22)	23 (22)	0 (71)	17 (71)
Bt Crystals: HD1 (10µg/ml)	0 (43)	0 (43)	0 (70)	0 (70)
Bt Spores: HD1 (0.5 D.O)	13 (22)	0 (22)	9 (22)	0 (22)
Crystals : spores (50:50)	26 (31)	0 (37)	0 (24)	0 (24)
Control (H_2O)	0 (21)	0 (21)	0 (46)	0 (46)

n= total number of treated larvae.

No effect of entomopathogenes inoculation was found on subletally infected larvae, since only those viruses to which *S. exigua* is a permissive species MbNPV and SeMNPV achieved the 64 and 17% of NPV mortality respectively. However numerous studies have reported so far the triggering effect of heterologous inoculation in activation of occult NPVs infections, including *S. exigua* (Murillo *et al*., 2011).

Reactivation of SeMNPV by chemical stressors in field conditions

Four instar larvae collected from plants treated with the stress factors copper sulfate and sodium selenate showed very low levels of NPV-induce mortality (Table 2). A Bt-based pesticide was included as a control since this pesticides used is highly extended in biological control crop- systems in Almeria. Environmental conditions or the actual ingested dose might have been affected this result.

Table 2: NPV-induced mortality for chemical activators and the Bt-based insecticide and water control in covertly infected *S. exigua* larvae treated in greenhouses.

Treatment	**% Mortalidad por NPV (n)**
Control	0 (71)
Bt-based pesticide (0.3 g/l)	2.8 (72)
Copper Sulfate (0.1%)	1.4 (74)
Sodium Selenate (1 ppm)	2.4 (82)

n= total number of treated larvae.

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